Biology of the Ectoparasitoid Wasp Agrothereutes lanceolatus (Hymenoptera : Ichineumonidae) : Host Acceptance and Larval Development on a Laboratory Host

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Biology of the Ectoparasitoid Wasp *Agrothereutes lanceolatus* (Hymenoptera: Ichneumonidae): Host Acceptance and Larval Development on a Laboratory Host

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Agrothereutes lanceolatus is a common parasitoid attacking several woodland and orchard lepidopteran pests in Japan. This study was undertaken to examine development of *A. lanceolatus* when *Galleria mellonella* was used as a laboratory host. Both wild–caught and laboratory–reared females readily accepted *G. mellonella* cocoons for attack and oviposition. The percentage successful parasitism was over 80%, suggesting that *G. mellonella* was a very suitable host for *A. lanceolatus*. The parasitoid was a solitary species: Under superparasitism lethal competition among early instars took place, and only one parasitoid developed to an adulthood, although two parasitoid offspring emerged in some exceptional cases. Female *A. lanceolatus* also accepted host cocoons containing pupae of the parasitoid *Pimpla nipponica* (Ichneumonidae) for oviposition, and the offspring developed successfully as a hyperparasitoid. This suggested that *A. lanceolatus* was a facultative hyperparasitoid.

INTRODUCTION

Agrothereutes lanceolatus Walker is a parasitic wasp distributed in East Asia (Townes *et al.*, 1965). It belongs to the subfamiliy Phygadeuontinae of Ichneumonidae (Hymenoptera). *A. lanceolatus* is known to attack mature larvae, prepupae and pupae of several tortoricids and pyralids (Lepidoptera), including some important pests such as *Chilo suppressalis* Walker, *Glyphodes pyloalis* Walker, and *Homona magnanima* Diakonoff (Yasumatsu and Watanabe, 1964; Townes *et al.*, 1965; Mao and Kunimi, 1991; Ueno, 1999). Given that *A. lanceolatus* is a very common parasitoid throughout Japan, it may play an important role in suppressing such lepidopteran pests. However, the potential of *A. lanceolatus* as a natural enemy of these lepidopterous pests is not known. General biology of *A. lanceolatus* also has poorly been investigated so far (Ueno, 1999).

This study is one of a series dealing with bionomics of *A. lanceolatus* and concerns host acceptance and larval development of *A. lanceolatus*. Specifically, experiments were conducted to determine development mode and time of *A. lanceolatus* in the laboratory. Examination of developmental characteristics of the parasitoid would provide essential information about the basic biology of *A. lanceolatus*.

Suitability of a factitious host was also assessed for rearing of *A. lanceolatus*. Given that natural hosts of *A. lanceolatus* are difficult to mass–rear in the laboratory, use of a factitious host would be ideal for rearing the parasitoid. Therefore, in this study, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) was used as a candidate laboratory host. *G.*

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mellonella was selected because it is easy to mass–rear and because it is known as a suitable laboratory host for a number of ichneumonid parasitoids (e.g. Sandlan, 1982; Ueno and Tanaka, 1994). Thus, acceptability of *G. mellonella* for *A. lanceolatus* and parasitoid offspring survival on *G. mellonella* were examined to assess host suitability.

MATERIALS AND METHODS

Insect cultures and general methods

A culture of *A. lanceolatus* was established in the laboratory from adult parasitoids collected in the campus of Nagoya University, central Japan and Fukuoka city, Kyushu, Japan. Cocoons containing prepupae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) were used as a laboratory host to rear the parasitoid.

Newly emerged female parasitoids were placed in plastic cups (10 cm in diameter, 4.5 cm in height) and were paired with males. Adult parasitoids were provided with a honey solution in tissue papers placed in the cups, and the papers were replaced twice a week. The colony was maintained at constant 20 °C and a photoperiod of 16L:8D.

For all experiments, laboratory–reared female parasitoids (F2 or F3) were selected for similarity in size and were 6–7 days old unless otherwise stated. *G. mellonella* larvae were reared on an artificial diet at 30–33 °C, and the cocoons containing prepupae were used in experiments.

Host acceptability

The aim of this experiment was to examine whether *G. mellonella* was accepted as host. For this purpose, wild–caught female *A. lanceolatus* was provided with *G. mellonella* cocoons, and females' response to the cocoon was observed. Response by laboratory–reared female parasitoids was also observed. Parasitoids collected at Nagoya and Fukuoka (see above) were used for testing. The age of wild–caught females was unknown, and they were used for testing 2–3 days after collection. One day before test, both wild–caught and laboratory–reared females were allowed access two host cocoons for 30 min.

Each female parasitoid in the cup was provided with two host cocoons, and the behavioral response was continuously observed. When a female encountered a host cocoon, her response was carefully observed and noted. When hosts were attacked, whether a female oviposited on, or rejected, a host after drilling into the host was confirmed by observing the base of her ovipositor under a binocular microscope. It is possible to see an egg passing through the base of the ovipositor of *A. lanceolatus* during oviposition under a binocular microscope, as with other ichneumonid wasps (Quicke, 1997). Hosts attacked by *A. lanceolatus* were dissected to examine where eggs were placed (i.e. on host body surface or within host body).

Larval development

This experiment was designed to examine offspring parasitoid development. Host cocoons were singly offered for female parasitoids and were observed for single parasitism. Parasitized hosts were collected and were placed singly in plastic cups (4.5 cm in diameter, 3.0 cm in height). The cups were kept at constant 25 °C and a photoperiod of

16L:8D. Daily observations were made at 24 h intervals to check the day of emergence of parasitoid offspring.

Superparasitism and larval competition

This experiment was conducted to examine the results of multiple egg deposition on single host individuals in parasitoid development. Fresh host cocoons were singly presented to female parasitoids for oviposition. After single oviposition on each host cocoon had been confirmed, the cocoon was removed and was presented to another female parasitoid for superparasitism after 1–3 min of the first parasitism. When the second oviposition (=superparasitism) was confirmed, host cocoons superparasitized were used for the experiment. Superparasitized hosts were placed singly in plastic cups (4.5 cm in diameter, 3.0 cm in height) and were kept at $25 \pm 0.5\,°C$ with a photoperiod of 16L:8D for parasitoid emergence. The day that parasitoids emerged was recorded. Hosts were kept up to 20–25 days since parasitism, and those that had not produced any parasitoids were dissected under a binocular microscope.

Facultative hyperparasitism

Test was conducted to assess whether *A. lanceolatus* could be a facultative hyperparasitoid. For this purpose, *G. mellonella* cocoons parasitized by a primary parasitoid were offered to female *A. lanceolatus* and its response to the cocoons was observed. When cocoons were hyperparasitized, the cocoons were reared as mentioned above.

As a primary parasitoid, the solitary pupal endoparasitoid *Pimpla nipponica* Uchida (Ichneumonidae), which is commonly found to coexist with *A. lanceolatus*, was used. Host cocoons containing pupae were allowed to be parasitized by *P. nipponica*, and were used for testing when *P. nipponica* was at the pupal stage.

Data analysis

Analysis of variance (ANOVA) was applied to examine effect of the parasitoid sex on parasitoid development time. Other data were analyzed with chi–squared tests. JMP (SAS Institute, 1997) was used for statistical analyses.

RESULTS AND DISCUSSION

Host acceptability

Because natural hosts used by *A. lanceolatus* are difficult to mass–rear, an alternative host for laboratory–rearing of *A. lanceolatus* is required. In the present study, *G. mellonella* was used as a candidate laboratory host, testing its suitability for rearing the parasitoid. The results demonstrated that it was readily accepted by *A. lanceolatus* for attack and oviposition (Table 1).

Wild–caught *A. lanceolatus* recognized and attacked host cocoons presented. Host cocoons once attacked were mostly parasitized (Table 1), and each parasitized host received only one parasitoid egg. Parasitoid eggs were found on the surface of host prepupae within the cocoons. Occasionally hosts were used for host–feeding.

Likewise, most laboratory–reared females accepted *G. mellonella* cocoons for oviposition (Table 1). Female *A. lanceolatus* laid one egg on each host. No difference

Table 1. Acceptability of a factitious laboratory host, *G. mellonella*, for wild–caught
and laboratory–reared *A. lanceolatus*

Generation	Strain	N of females	% attacked*	% oviposited*
Wild–caught	Nagoya	12	87.5	100
Reared	Nagoya	20	97.5	92.3
Wild–caught	Fukuoka	7	100	92.9
Reared	Fukuoka	13	100	96.2

* % of hosts attacked=Number of hosts probed with the ovipositor/Total number of
hosts given to each female parasitoid. % of hosts oviposited=Number of hosts
oviposited/Number of hosts attacked by female parasitoids. No significant
difference was detected in the percentages of hosts attacked or oviposited
(chi–squared test, $P > 0.05$). All parasitized (=oviposited) hosts received one egg
with no exception.

was found in the percentages attack and oviposition depending on parasitoid generations
and localities from which *A. lanceolatus* was originated (Table 1).

Although *G. mellonella* was not a natural host for *A. lanceolatus*, the female readily
recognized and attacked it. This may be because *G. mellonella* cocoons share some of
host cues that are used by *A. lanceolatus* for host recognition. A likely cue that is in
common could be the morphology of 'cocoon'. Parasitoids use a combination of different
cues to recognize their host, and host physical features are among the cues (Vinson,
1976; Arthur, 1981; Quicke, 1997).

Some ichneumonid parasitoids of Lepidoptera attack artificial hosts that morphologi-
cally mimic insect 'cocoons' (e.g. Henaut and Guerdoux, 1982). Likewise, the ichneu-
monid *A. lanceolatus* shows an interest in a white paper tube or cotton pad, which
mimics a 'cocoon', and often probes such a tube or pad with the ovipositor (Ueno, unpub-
lished). This suggests that *A. lanceolatus* uses at least in part physical cues that are in
common among insect cocoons, to recognize the host. Although *G. mellonella* is a factiti-
ous host, the morphology of the cocoons itself may cause ovipositor probing by *A.
lanceolatus*.

Larval development

Parasitized *G. mellonella* cocoons mostly produced offspring *A. lanceolatus* (para-
sitoid production > 80%) indicating that *G. mellonella* was highly suitable for *A.
lanceolatus* larval development (Table 2). With no exception, *A. lanceolatus* developed

Table 2. Host suitability of a factitious laboratory host, *G. mellonella*, for *A. lanceolatus*

Strain	N of hosts	% hosts producing parasitoids*	Developmental mode of parasitoids
Nagoya	108	80.6	Ectoparasitic
Fukuoka	56	83.9	Ectoparasitic

* No significant difference was detected in the percentages of hosts producing
parasitoid offspring (=offspring survival) between the strains of *A. lanceolatus*
(chi–squared test, $P > 0.05$).

as an ectoparasitoid, feeding on host prepupae from external positions of the hosts (Table 2).

The results showed that a factitious host, *G. mellonella*, is highly suitable for rearing of *A. lanceolatus* in terms of acceptability to the adult female (Table 1) and offspring production (Table 2). *G. mellonella* is widely recognized as a good laboratory host for rearing a number of parasitoids (e.g. Sandlan, 1982; Ueno and Tanaka, 1994), and this is the case for *A. lanceolatus*. The results also have confirmed that *A. lanceolatus* is an ectoparasitoid wasp like other members of Phygadeuontinae of Ichneumonidae.

Male wasps had a mean development time of 14.70 ± 0.17 ($\pm$SE) days with values ranging 13 to 19 days. Female development time ranged from 15 to 18 days with a mean of 16.12 ± 0.18 ($\pm$SE) days. ANOVA revealed that males developed significantly faster than females (N=70, df=2, F=31.10, $P<0.0001$) (Table 3). Faster development in male parasitoids than females has widely been reported for many parasitoids (Ueno and Tanaka, 1994; Quicke, 1997).

Table 3. Development time of male and female *A. lanceolatus* (Nagoya strain) when reared on *G. mellonella*

Sex of parasitoids	N	Development time (day)*
Male	37	14.7 ± 0.17
Female	33	16.1 ± 0.18

* Data were shown as mean $\pm$ SE. Females required significantly more time to complete the development (from egg to emergence) than males (ANOVA, P< 0.0001).

Superparasitism and larval competition

The results confirmed that *A. lanceolatus* was a solitary species (Table 4); even when two eggs were laid on a single host individual, only one parasitoid matured and developed to an adulthood. Offspring survival was not reduced due to superparasitism (Table 4). Dissection of superparasitized hosts after parasitoid emergence suggested that one immature parasitoid died mostly during its early stages of development (i.e. egg or first instar). Competition between two developing *A. lanceolatus* may occur within the early stages at least when superparasitism (i.e. the second parasitism) takes place shortly after the first parasitism. This pattern of larval competition is frequently observed for a number of solitary parasitoids (Mackauer, 1990; Quicke, 1997).

In solitary parasitoids, two or more immature parasitoids rarely developed to middle or late instars; only one winner of competition can develop to a middle instar or more, and losers died mostly during egg or first instar stage (Mackauer, 1990). In *A. lanceolatus*, however, there were four cases in which two middle instar larvae died. This suggested that scramble rather than context competition could occasionally occur, though context type competition during the early stage of development is the general rule.

In two cases, successful superparasitism was found; two parasitoid offspring emerged from a single host individual (Table 4). In endoparasitoid wasps successful superpara-

Table 4. Effect of superparasitism on production of parasitoid offspring in A. *lanceolatus*

Parasitism	N	% offspring survival*	% multiple parasitoid production**
Single	48	79.2	—
Superparasitism	68	75.0	3.9

* There was no significant difference in the percentages (chi–squared test, $P >$ 0.05).

** Percentage of hosts that produced two parasitoid offspring.

sitism is extremely rare while in ectoparasitoid wasps successful superparasitism occasionally takes place. Factors causing this difference are not known. Mechanism and process of competition might differ between ecto– and endoparasitoids.

Hyperparasitism

Members of the genus *Agrothereutes* belonging to Phygadeuontinae are known as primary ectoparasitoids, attacking principally Lepidoptera (Townes *et al.*, 1965). However, at least some of them may also be facultative hyperparasitoids (Shiotsu and Arakawa, 1982).

In the present paper, a laboratory experiment was conducted to examine whether *A. lanceolatus* could be a facultative hyperparasitoid. The experiment has revealed that *A. lanceolatus* attacks and oviposits on pupae of the primary endoparasitoid *Pimpla nipponica* within host pupae and is able to develop successfully (Table 5). This is the first experimental evidence that *Agrothereutes* species can be a hyperparasitoid of other hymenopterans. Although host records suggest this parasitoid is a primary parasitoid of Lepidoptera (Yasumatsu and Watanabe, 1964; Townes *et al.*, 1965), the potential host range could be wider due to facultative hyperparasitism.

Table 5. Facultative hyperparasitism by *A. lanceolatus*

Host	N	% parasitized *	% offspring production *
G. *mellonella*	25	96.0	83.3
P. *nipponica***	23	82.6	73.7

* No significant difference was detected between the groups (chi–squared test, $P >$ 0.05).

** Pupa of the ichneumonid endoparasitoid *P. nipponica* within *G. mellonella* pupa enclosed in the cocoon was used for testing.

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